

RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

Controlled fluorescence in a beetle's photonic structure and its sensitivity to environmentally induced changes

Mouchet, Sébastien R.; Lobet, Michaël; Kolaric, Branko; Kaczmarek, Anna M.; van Deun, Rik; Vukusic, Peter; Deparis, Olivier; Van Hooijdonk, Eloise

Published in: Proceedings of the Royal Society B: Biological Sciences

DOI: 10.1098/rspb.2016.2334 10.1098/rspb.2016.2334

Publication date: 2016

Link to publication

Citation for pulished version (HARVARD):

Mouchet, SR, Lobet, M, Kolaric, B, Kaczmarek, AM, van Deun, R, Vukusic, P, Deparis, O & Van Hooijdonk, E 2016. 'Controlled fluorescence in a beetle's photonic structure and its sensitivity to environmentally induced changes', Proceedings of the Royal Society B: Biological Sciences, vol. 283, no. 1845, 20162334. https://doi.org/10.1098/rspb.2016.2334, https://doi.org/10.1098/rspb.2016.2334

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
 You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

PROCEEDINGS B

Controlled fluorescence in a beetle's photonic structure and its sensitivity to environmentally induced changes

Journal:	Proceedings B	
Manuscript ID	RSPB-2016-2334.R1	
Article Type:	Research	
Date Submitted by the Author:	14-Nov-2016	
Complete List of Authors:	Mouchet, Sébastien; University of Exeter, School of Physics; Universite de Namur, Department of Physics Lobet, Michaël; Universite de Namur, Department of Physics Kolaric, Branko; Universite de Namur, Department of Physics; University of Mons, Currently with Micro- and Nanophotonic Materials Group, Faculty of Science Kaczmarek, Anna; Universiteit Gent, L3 – Luminescent Lanthanide Lab, Department of Inorganic & Physical Chemistry Van Deun, Rik; Universiteit Gent, L3 – Luminescent Lanthanide Lab, Department of Inorganic & Physical Chemistry Vukusic, Pete; University of Exeter, School of Physics Deparis, Olivier; Universite de Namur, Department of Physics Van Hooijdonk, Eloise; Universite de Namur, Department of Physics	
Subject:	Biophysics < BIOLOGY, Biomaterials < BIOLOGY	
Keywords:	Beetle scale, Fluorescence, Natural photonic crystal, Photonic bandgap materials, Structural colour, <i>Hoplia coerulea</i>	
Proceedings B category: Development & Physiology		

SCHOLARONE[™] Manuscripts

1 Title: Controlled fluorescence in a beetle's photonic structure and its sensitivity to 2 environmentally induced changes

3 4 5	Authors:	Sébastien R. Mouchet ^{1,2} , Michaël Lobet ¹ , Branko Kolaric ^{1,3} , Anna M. Kaczmarek ⁴ , Rik Van Deun ⁴ , Peter Vukusic ² , Olivier Deparis ¹ and Eloise Van Hooijdonk ¹			
6 7 9 10 11 12 13 14	Affiliations:	 ¹ Department of Physics, University of Namur, Rue de Bruxelles 61, 5000 Namur, Belgium ² School of Physics, University of Exeter, Stocker Road, Exeter EX4 4QL, United Kingdom ³ Currently with Micro- and Nanophotonic Materials Group, Faculty of Science, University of Mons, Place du Parc 20, B-7000 Mons, Belgi ⁴ L³ – Luminescent Lanthanide Lab, Department of Inorganic & Physical Chemistry, Ghent University, Krijgslaan 281-S3, B-9000 Ghent, Belgium 			
16 17 18 19	Email addresses:	s.mouchet@exeter.ac.uk, michael.lobet@unamur.be, branko.kolaric@unamur.be, anna.kaczmarek@ugent.be, rik.vandeun@ugent.be, p.vukusic@exeter.ac.uk, olivier.deparis@unamur.be, eloise.vanhooijdonk@unamur.be			
20 21	Contact details of the	e corresponding author:			
22 23	Sébastien R. Mouchet School of Physics				
24	University of Exeter				
25	Physics building Stocker Road				

- 25 Physics building, Stocker Road 26 Exeter EX4 4QL
- 27 United Kingdom
- 28 T. +44 (0)1392 724156
- 29 s.mouchet@exeter.ac.uk

30

31	CONTROLLED FLUORESCENCE IN A BEETLE'S
32	PHOTONIC STRUCTURE AND ITS SENSITIVITY TO
33	ENVIRONMENTALLY INDUCED CHANGES
34 35	Sébastien R. Mouchet ^{1,2} , Michaël Lobet ¹ , Branko Kolaric ^{1,3} , Anna M. Kaczmarek ⁴ , Rik Van Deun ⁴ , Peter Vukusic ² , Olivier Deparis ¹ and Eloise Van Hooijdonk ¹
36	¹ Department of Physics, University of Namur, Rue de Bruxelles 61, B-5000 Namur, Belgium
37	² School of Physics, University of Exeter, Stocker Road, Exeter EX4 4QL, United Kingdom
38 39	³ Currently with Micro- and Nanophotonic Materials Group, Faculty of Science, University of Mons, Place du Parc 20, B-7000 Mons, Belgium
40 41	⁴ L ³ – Luminescent Lanthanide Lab, Department of Inorganic & Physical Chemistry, Ghent University, Krijgslaan 281-S3, B-9000 Ghent, Belgium
42	The scales covering the elytra of the male Hoplia coerulea beetle contain fluorophores
43	embedded within a porous photonic structure. The photonic structure controls both
44	insect colour (reflected light) and fluorescence emission. Herein, the effects of water-
45	induced changes on the fluorescence emission from the beetle were investigated. The
46	fluorescence emission peak wavelength was observed to blue-shift on water
47	immersion of the elytra whereas its reflectance peak wavelength was observed to red-
48	shift. Time-resolved fluorescence measurements, together with optical simulations,
49	confirmed that the radiative emission is controlled by a naturally engineered photonic
50	bandgap while the elytra are in the dry state, whereas non-radiative relaxation
51	pathways dominate the emission response of wet elytra.
52	KEYWORDS: Beetle scale; fluorescence; natural photonic crystal; photonic bandgap materials;
53	structural colour
54	1. INTRODUCTION

55 Natural photonic structures such as those found in insects exhibit a large variety of optical 56 properties, among which structural colours (i.e. colours due to coherent scattering)¹⁻³, liquid-57 induced colour changes⁴⁻⁹ and colour sensitivity to gas or vapour¹⁰⁻¹⁵ have attracted much 58 interest. Many biological photonic structures are porous and comprise biopolymers such as 59 chitin, keratin and cellulose. The range of structures and optical effects found in biological 60 systems which have been optimised through evolution for millions of years, enables the 61 development of new designs and possible technological applications through an approach that incorporates bioinspired principles¹⁶⁻¹⁹. Another optical phenomenon found in living 62 63 organisms is fluorescence emission. This phenomenon consists of a process of radiative 64 decay (i.e. light emitting) of a substance that has previously been excited by absorption of 65 electromagnetic radiation of higher energy. Fluorescence is found in many living organisms, terrestrial as well as aquatic, including arthropods^{20,21} (e.g. butterflies²²⁻²⁵, beetles^{21,26}, 66 67 scorpions²⁷), marine invertebrates (e.g. corals²⁸, sea anemones²⁹), birds (such as parrots³⁰ and penguins³¹), plants³² as well as mammals³³ (e.g. tooth enamel, white hair and nails). 68 69 These organisms emit visible light and thus display colours when they are illuminated by 70 ultraviolet (UV) light. This light emission arises due to the presence of fluorophores, such as 71 biopterin or papilochrome II. Colour emission through fluorescence can range from blue, green, yellow to red²¹ depending on the fluorophores. 72

73 The confinement of fluorophores within photonic structures can lead to controlled 74 fluorescence, through modification of the system's density of optical states (DOS)³⁴⁻³⁷. When fluorescence occurs within the photonic bandgap (PhBG) of a photonic structure, a decrease 75 76 in the emission intensity is observed. This arises as a consequence of the associated increase in decay time τ of the excited states³⁴⁻³⁸. This sort of photonic confinement can be 77 found in several living organisms^{24,25,39-48}. We note that the contribution of fluorescence 78 79 emission to the colour appearance of a living organism is not always striking, often because, 80 available solar UV intensity and insect fluorophore internal quantum efficiency can be low. 81 This was highlighted in several *nireus* group butterflies (*Papilio bromius*, *Papilio epiphorbas*, Papilio nireus and Papilio oribazus) for which the contribution of the fluorescent blue 82 emission to their colour is minor²⁵. Fluorescence emission in living organisms is not 83 84 necessarily always functional. There is no known purpose, for instance, for the fluorescence of mammalian nails or tooth enamel. In contrast, however, the absorption of UV by fluorophores can provide insect species with protection against potential damage¹.

87 The male Hoplia coerulea (Drury 1773), a beetle from the family Scarabaeidae, exhibits a variety of optical properties including vivid iridescent colour^{2,49,50}, liquid- and vapour-induced 88 colour changes^{6,8,14,15,51} and fluorescence⁴³. The source of all these properties lies in the flat 89 90 circular scales covering the beetle's elytra. Each scale exhibits a bright blue iridescent colour^{2,49,50} (Figure 1a) under incident white light due to its more or less ordered 91 92 macroporous photonic structure. This structure can be described as a periodic stacked combination of thin pure cuticle layers and mixed air-cuticle porous layers⁵⁰ (Figure 1b-c). In 93 94 the dry state, these give rise to a Bragg reflectance peak in the blue part of the visible 95 spectrum (at approximately 460 nm at normal incidence). The wavelength of this peak blueshifts as incidence angle increases. When the insect is in contact with liquids^{6,8,51} or 96 vapour^{14,15}, its colour reversibly changes from blue to green, as a consequence of the fluids 97 98 penetrating within the photonic structure and inducing changes in refractive index contrast^{6,8,14,15,51}. One interesting aspect of this fluid-induced colour change is that it takes 99 100 place in a photonic structure that is not directly open to the surrounding environment⁸. An 101 envelope encases this photonic structure and mediates fluid exchanges with the 102 environment. Due to similarities with typical biological cells, this H. coerulea photonic 103 structure was previously referred to as a "photonic cell"⁸. Moreover, fluorophores are embedded within the structure. In other work⁴³ it was demonstrated that the confinement of 104 105 fluorescent sources in the modelled photonic structure of the scales gave rise both to enhancement and inhibition of the fluorescent emission at particular wavelengths. 106

107 Although there have been a few studies of liquid-induced fluorescence changes in insects' 108 photonic structures, specifically relating to three butterflies (*Morpho sulkowskyi*⁴⁷, *Papilio* 109 $zalmoxis^{47}$ and *P. nireus*²³), the area is very much under-explored. In these previously 110 reported studies^{23,47}, a liquid with a refractive index close to chitin was used to remove the 111 effects of the photonic structure on the fluorescence steady state, by index matching. Significant decreases in the emitted energy⁴⁷ and the decay time²³ were observed with the decrease of the refractive index contrast, while variations of the emission peak wavelength as function of refractive index change were rather small (blue-shift of less than 10 nm)⁴⁷.

In this work, changes in the fluorescence steady state of the fluorophores located in the elytra of male *H. coerulea* beetles, upon contact with water, were experimentally observed. This led to a blue-shift of the fluorescent emission from the insect structure, a feature that was previously unnoticed. The fluorescence-associated colour changed from turquoise (bluegreen) to dark blue. Using several morphological and optical characterisation techniques in addition to optical simulations, this surprising response was explained in terms of waterinduced changes of the photonic environment in the scales' porous structure.

122 2. MATERIALS AND METHODS

123 (A) PHOTONIC STRUCTURE MORPHOLOGY

The morphology of the elytra was investigated using a FEI Tecnai 10 (Hillsboro, Oregon, 124 125 USA) transmission electron microscope (TEM) and a FEI Nova Nanolab 200 Dual-Beam (Hillsboro, Oregon, USA) scanning electron microscope (SEM). Elytra of dead H. coerulea 126 were prepared following a standard sample preparation method⁵². 100 nm-thick cross 127 128 sections were ultramicrotomed and transferred onto TEM analysis grids. For SEM analysis, elytra were cut into pieces of about $5 \times 5 \text{ mm}^2$ and attached to the sample mount by 129 130 conducting adhesive tape. This was sputter-coated with 20 nm of platinum. The focussed-ion 131 beam facility (FIB) on the FEI Nova Nanolab 200 Dual-Beam SEM was used to reconstruct a 132 three dimensional representation of the scale structure (FEI Avizo 3D Software).

133 (B) OPTICAL CHARACTERISATION

Optical microscopy was performed using an Olympus BX61 (Tokyo, Japan) microscope, an Olympus XC50 camera and an Olympus BX-UCB visible light source (in reflection mode) or a Lumen Dynamics X-cite Series 120PCQ (Mississauga, Ontario, Canada) UV-lamp (in
 fluorescence mode). Further details are available as supplementary material.

The normalised reflection spectra R = (I - B)/(W - B), i.e. the ratio between the spectral intensities *I* and *W* reflected by the sample and by an Avantes WS-2 (Apeldoorn, The Netherlands) white reference, respectively, including noise corrections *B*, were measured using an Ocean Optics QE65Pro (Dunedin, Florida, USA) spectrophotometer connected to the microscope. The numerical aperture of the microscope objective was equal to 0.50. The use of a microspectrophotometer allowed us to analyse very small areas of the elytra comprising only a few scales (i.e. spot sizes of approximately 30 µm diameter).

Fluorescence measurements were performed using an Edinburgh Instruments (Livingston, UK) FLSP920 UV-vis-NIR spectrofluorimeter equipped with a Hamamatsu R928P (Hamamatsu City, Japan) photomultiplier-tube. The recorded time-resolved dynamics were fitted by single exponential functions. Only in the case of the dry sample within the PhBG (at 466 nm), the best fit was obtained using a double exponential function. These closest theoretical fits enabled us to determine the decay time of the fluorescence emission. Further details regarding spectrofluorimetry measurements are available as supplementary material.

The chemistry of *H. coerulea's* fluorophores has not yet been identified. Furthermore, the distribution of this pigment in the photonic structure has not yet been experimentally determined. More detailed investigations of the fluorophores are necessary and are beyond the scope of the present study.

156 (C) PHOTONIC MODEL OF THE BEETLE SCALE AND NUMERICAL METHODS

The elytra of male *H. coerulea* beetles are covered by almost circular scales, composed principally of chitin. Their average diameter is approximately 80 µm and their thickness is approximately 3.5 µm. The photonic structure responsible for the specular reflection of light at these scales' surfaces is revealed by electron microscope images (Figure 1b-c). It is a porous multilayer formed by the periodic stacking of thin, flat pure cuticle layers and thick
 mixed air-cuticle porous layers (network of rods separated by air gaps).

Based on similar electron microscope images, Vigneron *et al.*⁵⁰ and Rassart *et al.*⁶ identified the geometrical parameters of the structure. In our study, we used the same photonic model as was presented in the two earlier studies^{6,50}. On average, 12 bilayers are found in the periodic stack (Figure 1b-d). Vigneron and Rassart give the thickness of cuticle layers as 35 nm and the thickness of the mixed air-cuticle layers as 140 nm. Their stated width of the rods is 90 nm and the air gap between two successive rods is 85 nm.

The refractive index n_{chitin} of cuticle material (mainly chitin) is often quoted as equal to 1.56 in 169 the visible range⁵³. This average dispersionless value is a good trade-off between dispersion 170 relations found in the literature⁵⁴⁻⁵⁶ for butterfly scales and beetle exocuticle. These relations 171 172 are valid only in the visible range whereas the refractive index of cuticle material in the near-173 UV range is actually not known. As first approximation, the same refractive index value was 174 used in all our simulations from near-UV to visible ranges. In the dry state, the mixed air-175 cuticle layers are approximated by a homogeneous material with an effective refractive index n_{mixed} lying between 1 (air) and 1.56 (chitin). Using a previously reported effective medium 176 approximation⁶, a value of $n_{\text{mixed}} = 1.26$ is calculated for the mixed air-cuticle layers. The 177 178 modelled photonic structure therefore consists of a 1D periodic stack of thin pure cuticle 179 layers and thicker effective layers (Figure 1d). In the wet state, since water ($n_{water} = 1.33$) replaces air in pores, the effective refractive index becomes $n_{\text{mixed}} = 1.44$. Using an effective 180 181 medium approximation is justified since the photonic structure does not give rise to non zeroorder diffraction at visible wavelengths⁵⁰ as a result of the disorder in the orientations of the 182 183 rods and the small distances between them (i.e. 175 nm).

A conventional one-dimensional transfer-matrix (1D-TM) method approach⁵⁷ was used to simulate reflectance spectra of the *H. coerulea* multilayer structure in dry and wet states. This method rigorously solves Maxwell's equations in each layer of the photonic structure for 187 the propagation of electromagnetic waves through layered media. In this formalism, the 188 electromagnetic field wave is decomposed in each layer into forward and backward waves 189 propagating in the direction perpendicular to the layers. An extension of the 1D-TM method^{43,47} was employed in order to model light emission from the structure. This extended 190 191 method relies on the calculation of spectral variations in the emitted intensity, normalised 192 with respect to a source in free space. It requires light to be homogeneously emitted by a 193 single layer, in which a non-zero current density vector is included in Maxwell's equations in 194 order to represent a uniform distribution of fluorophores. For a more realistic simulation of 195 arbitrary fluorophore distribution, emission spectra were calculated with the emission source 196 located in each pure cuticle layer separately, and then averaged in order to simulate light 197 emission by the sources (fluorophores) distributed across the whole photonic structure.

Simulations were also performed using the finite-difference time-domain (FDTD) method⁵⁸,
 using the MIT Electromagnetic Equation Propagation (MEEP 1.2) package⁵⁹. Further details
 regarding these simulations are available as supplementary material.

201 The photonic band structure and the Density Of optical States (DOS) were calculated in the 202 specific case of an infinite 1D photonic crystal based on the H. coerulea photonic structure 203 using a Kronig-Penney model approach and presented in ref. 60. A frequency-domain 204 method, based on an eigensolver for Maxwell's equations in a plane wave basis, was used to compute the Local Density Of optical States (LDOS)⁶¹. The LDOS $N_{\text{LDOS}}(\vec{r},\omega)$ counts the 205 available number of electromagnetic modes in which photons can be emitted at the specific 206 location of the emitting source. It depends therefore on the frequency ω and the position \vec{r} 207 208 of the emitting source in the environment but not on the propagation direction. It is known to be related to the emitter decay time (according to Fermi's golden rule) by the relation: 209 $\frac{\tau_0}{\tau} = \frac{N_{\text{LDOS}}(\vec{r}, \omega)}{N_{\text{LDOS}}(\vec{r})}$ where τ_0 is the decay time of one emitter located in free space, τ is the 210

211 decay time of the emitter and $N_{\text{LDOS},0}(\vec{r})$ is the LDOS of the emitter in free space. When

212 $N_{\text{LDOS}}(\vec{r}, \omega)$ is equal to zero, no propagation mode is available at position \vec{r} and frequency 213 ω . In this case, the decay time τ is infinite and light emission is inhibited.

The same structural model was used in all simulation methods. However, for calculations of the photonic band structure, namely DOS and LDOS, the number of bilayers was assumed to be infinite instead of equal to 12.

217 3. RESULTS AND DISCUSSION

The colour displayed by the male *H. coerulea* beetle scales is violet-blue (Figure 2a) and turns to green when they are in contact with water (Figure 2b). This arises due to the filling of the scales' macropores with water⁶. This appearance change corresponds to the shift of the reflectance peak maximum from 458 nm to 525 nm (namely, a red-shift) (Figure 2c). Decreases in reflectance intensity as well as in peak reflectance width are also observed.

223 Under exposure to UV light, the fluorescence emission from the H. coerulea elytra changes 224 from turquoise to dark blue upon contact with water (Figure 2d-e). The main features of the 225 excitation spectrum are consistent (Figure 2f): the peak wavelength is found at 365 nm and 226 367 nm in the dry and wet states, respectively, and their associated full width at half 227 maximum (FWHM) values are equal to 72 nm and 67 nm, respectively. Excitation of the 228 fluorophores is not influenced by contact with water. This indicates that the observed liquid-229 induced changes do not affect the ground states of the fluorophores. However, clearly the 230 emission spectrum of the scales is significantly modified (Figure 2f): they exhibit an 231 immersion-mediated blue-shift from 463 nm to 446 nm ($\Delta\lambda = 17 \,\mathrm{nm}$) and a decrease in FWHM from 121 nm to 105 nm. This response largely exceeds the responses measured in 232 the cases of butterfly species (typically of less than 10 nm)⁴⁷. Notably, the direction of the 233 234 immersion-mediated change of fluorescence emission peak wavelength is opposite to that of 235 the immersion-mediated shift of reflectance peak wavelength.

The water-induced changes in fluorescence emission were found to be reversible, a property also associated with the changes in reflectance. This infers that the fluorophores are not significantly altered chemically by exposure to water and UV under our experimental conditions. For both excitation and emission spectra, a decrease in intensity upon contact with water is observed (Figure 2f). It can be explained by, among other processes, the presence of water at the surface of the sample modifying light scattering efficiency.

242 If fluorophores are located in an infinite photonic crystal with a significant refractive index 243 contrast, as well as a complete PhBG preventing emission, and provided their fluorescence 244 efficiency is assumed to be equal to 1 (namely, the only decay process is fluorescence), they 245 will remain in their excited states. However, in the case of a finite crystal, or one with a low 246 refractive index contrast, the decay will be radiative with an associated decay time longer 247 than in free space. In order to investigate the effect of the environment on the fluorescence 248 emission further, time-resolved measurements were performed. Data from these 249 measurements indicate that when the elytron is in the dry state and has an emission 250 wavelength inside the PhBG (at 466 nm), the decay time τ (Table 1) is significantly longer 251 (3.9 ns) than for a wet elytron (1.4 ns). Outside the PhBG (at 546 nm), regardless of the wet 252 or dry state, τ is shorter (1.9 ns and 1.4 ns in the dry and wet states, respectively). In the 253 case of the wet state, the decay time of the fluorescence emission is the same both inside 254 and outside the PhBG. This may be partly explained by the decrease in the system's 255 refractive index contrast that leads to an associated decrease of the fluorescence inhibition in the wet state⁶². However, combined with the experimentally measured decrease in 256 257 fluorescence emission intensity, this observation clearly indicates that the wet state opens 258 non-radiative relaxation pathways, for instance guenching processes, that in parallel 259 decrease the effect of the optical system's PhBG on fluorescence emission. In contrast, in 260 the dry state the presence of the PhBG strongly influences emission properties and causes 261 an increase in fluorescence emission decay time as well as a double exponential decay. The 262 inhibition of fluorescence emission related to the observed increase of decay time is

http://mc.manuscriptcentral.com/prsb

263 explained by a lack of available modes for the radiative decay of the fluorophores embedded 264 within the photonic structure with respect to the same fluorophores in a homogeneous 265 medium. Due to this inhibition, a redistribution of energy has hence to take place leading to non-radiative transfers to the environment³⁸. The longest decay time (3.9 ns) corresponds to 266 the real life time of the fluorophores within the photonic structure. The shortest decay time 267 (0.79 ns) is related to non-radiative relaxations of the excited states. The time-resolved 268 269 measurements of the fluorescence emission from probes embedded within colloidal photonic structures^{37,38} are perfectly in agreement with these results. We observed that the decay time 270 271 in the dry state is more than twice the value of the decay time in the wet state, or at a 272 frequency outside the PhBG. This is counterintuitive if we take into account the low refractive 273 index contrast of the materials forming the photonic structure. It is a result that may be 274 explained at a phenomenological level by considering the curvature of the biological photonic 275 structure: this can additionally alter the system's photonic properties and, therefore, its fluorescence emission^{63,64}. 276

277 In the simulated photonic band structure (Figure 3b) and related DOS (Figure 3c), first and 278 second order PhBGs are predicted at 231 nm and 464 nm in the dry state. Despite the 279 presence of these two PhBGs, fluorescence emission can arise because the excitation peak 280 wavelength (Figure 2f) is located between these two PhBGs: the experimental excitation 281 peak wavelength was measured at approximately 365 nm. Additionally, the dry sample's reflectance peak wavelength, at 45° incidence (i.e. the same angle as was used for the 282 283 emission spectra measurements) was 436 nm. This reflectance peak presented a 79 nm 284 FWHM. Furthermore, the simulated reflectance spectra (at normal incidence) in dry and wet 285 conditions (Figure 3a) confirmed the experimentally-measured red-shift that is induced by 286 contact with water. Namely, the calculated reflectance peak wavelength is 461 nm in the dry 287 state and 501 nm in the wet state. The PhBGs also shift towards longer wavelengths when 288 air is replaced by water in the macropores of the photonic structure and this is the 289 mechanism by which the decay time of the emission at 466 nm is modified. The decreases in

reflectance peak intensities and FWHMs, as well as the PhBG widths (in the visible and the UV ranges), are also predicted when water replaces air in the structure. These predicted decreases, in addition to the changes in reflectance peak wavelength, agree with the observed changes of the reflectance spectrum (Figure 2c) and previously reported observations⁶. Both decreases are explained by the decrease of the effective refractive index contrast n_{chitn} / n_{mixed} between the layers⁵⁹ from 1.24 (dry state) to 1.08 (wet state).

296 Differences between measured and simulated spectra can be observed (e.g. in terms of 297 peak wavelengths and widths). These, in part, may arise from systematic errors associated 298 with the experimental incidence and detection angles (for instance, a 20°-incidence leads to 299 a 16-nm blue-shift of the reflectance peak position with respect to a normal incidence). 300 Furthermore, although the incident beam width is smaller than the size of the scales, the 301 beam may have not been centred on one single scale: a few sections of different scales may 302 have been analysed concurrently. Finally, the system's photonic structure is modelled as an 303 idealised perfectly periodic system even though it exhibits irregular layer interfaces, 304 inhomogeneities in refractive indices and dimensions, etc.

305 The computed $\frac{\tau_0}{\tau} = \frac{N_{\text{LDOS}}(\vec{r}, \omega)}{N_{\text{LDOS},0}(\vec{r})}$ ratio turns out to be equal to zero inside the PhBG (e.g. at

306 466 nm) in the dry state regardless of the position of the emitter. This corresponds to an 307 infinite decay time τ , i.e. light emission is inhibited. However, in the wet state, this ratio 308 ranges from 0.66 (at the layer interfaces) and 0.86 (at the middle of the mixed air-cuticle 309 porous layers). Due to the water-induced shift of the PhBG, light can be emitted at this wavelength. Outside the PhBG (i.e. at 546 nm), $\frac{\tau_0}{\tau}$ ranges from 0.6 (at the middle of the 310 311 mixed air-cuticle porous layers) to 0.8 (at the layer interfaces) regardless of the state (dry or 312 wet) of the modelled photonic structure. These results confirm the measured decay time 313 variations induced by contact with water. We note that this ratio cannot be calculated from experimental data because the decay time τ_0 in free space of the particular fluorophores 314

embedded in the structure is unknown. In addition to the absence of defects assumed in the modelled photonic structure, we mention that the measurements were not performed on a single fluorescence source. Each fluorophore located in the analysis area influenced the measurement.

Since the photonic structure controls the fluorescence emission^{43,47}, the emission spectrum is 319 320 modified by the change in refractive index within the macropores of the photonic structure. In 321 order to demonstrate this effect, two models of fluorescent sources were investigated. In both 322 models, the fluorophores were assumed to be homogeneously distributed throughout the 323 cuticular material in the photonic structure. In the first set of models, fluorophores formed a 324 uniformly planar source (emitting a uniform spectrum and located at the position of one pure 325 cuticle layer). These simulations were performed for each of the 12 pure cuticle layer positions using the extended 1D-TM method and the resulting emission spectra were 326 327 averaged (Figure 4a). In the second model (computed using a FDTD method), fluorophores 328 were modelled by 180 point sources randomly located (according to a continuous uniform 329 distribution) across the cuticle material of structure (i.e., taking into account the filling fraction 330 of material in the pure cuticle and mixed air-cuticle porous layers) (Figure 4b). In both 331 models, the intensity emitted by the sources embedded in the structure was normalised to 332 the intensity emitted by the sources in the absence of the structure. In this way, normalised 333 values greater than unity are associated with enhancement of the fluorescence by the 334 photonic structure at the corresponding wavelengths (Figure 4). Similarly, values less than 335 unity are associated with inhibition. In principle, re-absorption can affect fluorescence, 336 especially in the case of high quantum yield fluorophores. If re-absorption (or other non-337 radiative processes) takes place, the decay of fluorescence intensity is modified and does 338 not follow a single exponential law any more. This is not the case here outside the PhBG, 339 where the decay of fluorescence intensity was found to follow a single exponential law 340 (Figure S1). The absence of substantial overlap between fluorescence excitation and 341 emission spectra and the observation of clean, single peaked emission spectra (Figure 2f) 342 also suggest that re-absorption is negligible here. Therefore, re-absorption was not taken into

http://mc.manuscriptcentral.com/prsb

343 account in our simulations. In both emitted intensity spectra, a blue-shift of emission peak 344 wavelengths (corresponding to strong enhancement of emission) occurs; the peak at 480 nm 345 blue-shifts to 428 nm and the peak at 429 nm shifts to 416 nm in the cases of first and 346 second models, respectively. Although both models are subject to unavoidable assumptions, 347 it is important to observe that simulations (based on two different methods) are in qualitative agreement. It is however important to admit that a perfect match cannot be expected since 348 349 the emitting sources are modelled in radically different ways. The common point is the 350 assumed uniformity of fluorophores distribution. The qualitative agreement between 351 simulations (Figure 4) and measurements (Figure 2f) suggests that the fluorophores are 352 distributed throughout the photonic structure.

353 **4. CONCLUSIONS**

354 We investigated the male H. coerulea beetle that presents a broad variety of optical 355 properties. Inside the scales that cover its elytra is a macroporous photonic architecture 356 responsible for structural colour and fluorophores responsible for fluorescence emission. Our 357 experiments revealed that the macroporous nature of this photonic system supports fluid-358 induced colour changes. H. coerulea's intra-scale photonic structure can be approximated by 359 a periodic multilayer stack comprising pure cuticle layers and mixed air-cuticle porous layers. 360 Despite this structure's PhBGs the optical system has evolved in such a way that 361 fluorescence emission is not inhibited, i.e. excitation wavelengths do not overlap with the 362 system's PhBGs. The influence of the system's exposure to water on its fluorescence 363 emission was also investigated for the first time. A 17 nm water-induced blue-shift, from 364 turquoise to dark blue, of the emission peak wavelength was measured. This contrasted to 365 the water-induced 67 nm red-shift of the reflectance peak wavelength. These changes arise 366 due to modification in effective refractive index following pore filling by water. An additional 367 consequence of contact with water is the decrease of the fluorescence decay time at a 368 wavelength inside the PhBG. In the dry state, this decay time is significantly longer inside, 369 compared to outside, the PhBG. This is the result of the PhBG's influence on fluorescence

http://mc.manuscriptcentral.com/prsb

370 emission. Since, in the wet state, the decay time is the same inside and outside, the PhBG, 371 we can conclude that guenching processes take place and non-radiative relaxation pathways 372 dominate the emission mechanism. Simulations of light emission from the system indicated 373 that the presence of water in the macropores of the photonic structure leads to a blue-shift of 374 the emission spectrum. This agrees with experimental data for the system. The simulations 375 additionally confirmed the likelihood of a homogeneous distribution of fluorophores across 376 the structure and the role of the multilayer in this water-induced change in fluorescence 377 emission. Such a photonic system offers a new possibility to design novel functional optical 378 materials and coatings in technological areas such as imaging, lighting, biosensing and solar 379 cells.

380 DATA ACCESSIBILITY.

381 Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.sm72f.

382 **COMPETING INTERESTS.**

383 We declare we have no competing interests.

384 **AUTHORS' CONTRIBUTIONS.**

385 S.R.M., M.L. and E.V.H. conceived the original project. S.R.M. performed the morphological 386 characterisation. S.R.M. and E.V.H. conducted the optical and fluorescence microscopy 387 analyses as well as the reflectance measurements. A.M.K. and S.R.M. performed the 388 fluorescence measurements. B.K. and S.R.M. performed the time-resolved fluorescence 389 data analysis. S.R.M. performed the LDOS simulations and M.L., the FDTD method 390 simulations. E.V.H. and S.R.M. performed the 1D-TM method simulations as well as the 391 calculation of the photonic band structures and related DOS. S.R.M., M.L., B.K., A.M.K., 392 R.V.D., P.V., O.D. and E.V.H. discussed the results. S.R.M., M.L. and E.V.H. wrote the 393 manuscript with input from B.K., P.V. and O.D. All authors commented on the manuscript 394 and gave approval to its final version.

395 ACKNOWLEDGEMENTS.

396 The authors thank Louis Dellieu (Department of Physics, UNamur) for technical support 397 during the collection of samples and Michaël Sarrazin (Department of Physics, UNamur) for 398 commenting an early version of this article as well as for fruitful discussions. This research 399 used resources of the "Plateforme Technologique de Calcul Intensif (PTCI)", UNamur 400 (http://www.ptci.unamur.be), which is supported by F.R.S.-FNRS under the convention No. 401 2.4520.11 as well as of the Electron Microscopy Service (SME), UNamur 402 (http://www.unamur.be/en/sevmel). PTCI and SME are members of the "Consortium des 403 Équipements de Calcul Intensif (CÉCI)" (http://www.ceci-hpc.be) and of the "Plateforme 404 Technologique Morphologie – Imagerie" (UNamur), respectively.

405 Funding.

S. R. Mouchet was supported by the Belgian National Fund for Scientific Research (F.R.S.-406 407 FNRS) as a Research Fellow and by Wallonia-Brussels International (WBI) through a 408 Postdoctoral Fellowship for Excellence program WBI.WORLD. B. Kolaric acknowledges 409 financial support from the "Action de Recherche Concertée" (BIOSTRUCT project -410 No.10/15-033) of UNamur, from Nanoscale Quantum Optics COST-MP1403 action and from 411 F.R.S.-FNRS; Interuniversity Attraction Pole: Photonics@be (P7-35, Belgian Science Policy 412 Offfice). A. M. Kaczmarek acknowledges Ghent University's Special Research Fund (BOF) 413 for a Postdoctoral Mandate (project BOF15/PDO/091). R. Van Deun thanks the Hercules Foundation (project AUGE/09/024 "Advanced Luminescence Setup") for funding. E. Van 414 415 Hooijdonk was supported by F.R.S.-FNRS as a Postdoctoral Researcher. This research was 416 also supported by F.R.S.-FNRS through the Researchers' Credit CC 1.5075.11F and the 417 Research Credit CDR J.0035.13.

418 **References**

419 1. Berthier S. 2000 La couleur des papillons ou l'impérative beauté - Propriétés optiques des
 420 ailes de papillons. Springer, Paris.

421 2. Berthier S. 2003 Iridescences, les couleurs physiques des insects. Springer, Paris.

422 3. Kinoshita S. 2008 Structural Colors in the Realm of Nature. World Scientific Publishing Co, 423 Singapore. 424 4. Vigneron JP, Pasteels JM, Windsor DM, Vértesy Z, Rassart M, Seldrum T, Dumont J, Deparis O, Lousse V, Biró LP et al. 2007 Switchable reflector in the Panamanian tortoise 425 426 beetle Charidotella egregia (Chrysomelidae: Cassidinae). Phys. Rev. E 76, 031907. (doi:10.1103/PhysRevE.76.031907) 427 428 5. Rassart M, Colomer JF, Tabarrant T, Vigneron JP. 2008 Diffractive hygrochromic effect in the cuticle of the hercules beetle Dynastes hercules. New J. Phys. 10, 033014. 429 430 (doi:10.1088/1367-2630/10/3/033014) 431 6. Rassart M, Simonis P, Bay A, Deparis O, Vigneron JP. 2009 Scales coloration change 432 following water absorption in the beetle Hoplia coerulea (Coleoptera). Phys. Rev. E 80, 433 031910. (doi:10.1103/PhysRevE.80.031910) 7. Liu F, Dong BQ, Liu XH, Zheng YM, Zi J. 2009 Structural color change in longhorn beetles 434 435 Tmesisternus isabellae. Opt. Express 17, 16183-16191. (doi:10.1364/OE.17.016183) 8. Mouchet SR, Van Hooijdonk E, Welch VL, Louette P, Colomer JF, Su BL, Deparis O. 2016 436 437 Liquid-induced colour change in a beetle: the concept of a photonic cell. Sci. Rep. 6, 19322. 438 (doi:10.1038/srep19322) 9. Wang W, Zhang W, Fang X, Huang Y, Liu Q, Gu J, Zhang D. 2014 Demonstration of 439 440 higher colour response with ambient refractive index in *Papilio blumei* as compared to 441 Morpho rhetenor. Sci. Rep. 4, 5591. (doi:10.1038/srep05591) 442 10. Potyrailo RA, Ghiradella H, Vertiatchikh A, Dovidenko K, Cournover JR, Olson E. 2007 443 Morpho butterfly wing scales demonstrate highly selective vapour response. Nat. Photonics 1, 123-128. (doi:10.1038/nphoton.2007.2) 444 445 11. Biró LP, Kertész K, Vértesy Z, Bálint Zs. 2008 Photonic nanoarchitectures occurring in 446 butterfly scales as selective gas/vapor sensors. Proc. SPIE 7057, 705706. 447 (doi:10.1117/12.794910) 448 12. Mouchet S, Deparis O, Vigneron JP. 2012 Unexplained high sensitivity of the reflectance 449 of porous natural photonic structures to the presence of gases and vapours in the 450 atmosphere. Proc. SPIE 8424, 842425. (doi:10.1117/12.921784) 451 13. Potyrailo RA, Starkey TA, Vukusic P, Ghiradella H, Vasudev M, Bunning T, Naik RR, 452 Tang Z, Larsen M, Deng T et al. 2013 Discovery of the surface polarity gradient on iridescent 453 *Morpho* butterfly scales reveals a mechanism of their selective vapor response. P. Natl Acad. 454 Sci. USA 110, 15567-15572. (doi:10.1073/pnas.1311196110) 455 14. Mouchet S, Su BL, Tabarrant T, Lucas S, Deparis O. Hoplia coerulea, a porous natural 456 photonic structure as template of optical vapour sensor. 2014 Proc. SPIE 9127, 91270U. 457 (doi:10.1117/12.2050409) 458 15. Mouchet SR, Tabarrant T, Lucas S, Su BL, Vukusic P, Deparis O. Vapor sensing with a 459 natural photonic cell. Opt. Express 24, 12267-12280. (doi:10.1364/OE.24.012267) 460 16. Biró LP, Vigneron JP. 2011 Photonic nanoarchitectures in butterflies and beetles: 461 valuable sources for bioinspiration. Laser Photonics Rev. 5, 27-51. 462 (doi:10.1002/lpor.200900018) 17. Kim JH, Moon JH, Lee SY, Park J. 2010 Biologically inspired humidity sensor based on 463 464 three-dimensional photonic crystals. Appl. Phys. Let. 97, 103701. (doi:10.1063/1.3486115) 18. Ghazzal MN, Deparis O, De Coninck J, Gaigneaux EM. 2013 Tailored refractive index of 465 inorganic mesoporous mixed-oxide Bragg stacks with bio-inspired hygrochromic optical 466 properties. J. Mater. Chem. C 1, 6202-6209. (doi:10.1039/c3tc31178c) 467 468 19. Deparis O, Ghazzal MN, Simonis P, Mouchet SR, Kebaili H, De Coninck J, Gaigneaux 469 EM, Vigneron JP. 2014 Theoretical condition for transparency in mesoporous layered optical 470 media: Application to switching of hygrochromic coatings. Appl. Phys. Lett. 104, 023704. 471 (doi:10.1063/1.4862658) 472 Lawrence RF. 1954 Fluorescence in arthropoda. J. Ent. Soc. South Africa 17, 167-170.

- 473 21. Welch VL, Van Hooijdonk E, Intrater N, Vigneron JP. 2012 Fluorescence in insects. Proc.
 474 SPIE 8480, 848004. (doi:10.1117/12.929547)
- 22. Cockayne EA. 1924 I. The Distribution of Fluorescent Pigments in Lepidoptera. T. Roy.
 Ent. Soc. London 72, 1-19. (doi:10.1111/j.1365-2311.1924.tb03347.x)
- 477 23. Vukusic P, Hooper I. 2005 Directionally Controlled Fluorescence Emission in Butterflies.
 478 Science 310, 1151. (doi:10.1126/science.1116612)
- 479 24. Trzeciak TM, Wilts BD, Stavenga DG, Vukusic P. 2012 Variable multilayer reflection 480 together with long-pass filtering pigment determines the wing coloration of papilionid 481 butterflies of the *nireus* group. Opt. Express 20, 8877-8890. (doi:10.1098/rsfs.2011.0082)
- 482 25. Wilts BD, Trzeciak TM, Vukusic P, Stavenga DG. 2012 Papiliochrome II pigment reduces
 483 the angle dependency of structural wing colouration in *nireus* group papilionids. J. Exp. Biol.
 484 215, 796-805. (doi:10.1242/jeb.060103)
- 485 26. Israelowitz M, Rizvi SHW, von Schroeder HP. 2007 Fluorescence of the "fire-chaser" 486 beetle, *Melanophila acuminate*. J. Lumin. 126, 149-154. (doi:10.1016/j.jlumin.2006.06.017)
- 487 27. Pavan M, Vachon M. 1954 Sur l'existence d'une substance fluorescente dans les 488 téguments des Scorpions (Arachnides). C. R. Acad. Sci. Paris 239, 1700-1702.
- 28. Catala-Stucki R. 1959 Fluorescence Effects from Corals irradiated with Ultra-Violet Rays.
 Nature 183, 949. (doi:10.1038/183949a0)
- 491 29. Phillips CES. 1927 Fluorescence of Sea Anemones. Nature 119, 747. 492 (doi:10.1038/119747c0)
- 493 30. Arnold KE, Owens IPF, Marshall NJ. 2002 Fluorescent Signaling in Parrots. Science 295,
 494 92. (doi:10.1126/science.295.5552.92)
- 495 31. McGraw KJ, Toomey MB, Nolan PM, Morehouse NI, Massaro M, Jouventin P. 2007 A
 496 description of unique fluorescent yellow pigments in penguin feathers. Pigm. Cell Res. 20,
 497 301-304. (doi:10.1111/j.1600-0749.2007.00386.x)
- 498 32. Goodwin RH. Fluorescent substances in plants. 1953 Annu. Rev. Plant. Physiol. 4, 283 304. (doi:10.1146/annurev.pp.04.060153.001435)
- 33. Tani K, Watari F, Uo M, Morita M. 2004 Fluorescent Properties of Porcelain-Restored
- 501 Teeth and Their Discrimination. Mater. Trans. 45, 1010-1014.
- 502 (doi:10.2320/matertrans.45.1010)
- 34. Purcell EM. Spontaneous emission probabilities at radio frequencies. 1946 Phys. Rev.
 69, 681. (doi:10.1103/PhysRev.69.674.2)
- 505 35. Yablonovitch E. 1987 Inhibited Spontaneous Emission in Solid-State Physics and 506 Electronics. Phys. Rev. Lett. 58, 2059-2062. (doi:10.1103/PhysRevLett.58.2059)
- 507 36. John S. 1987 Strong Localization of Photons in Certain Disordered Dielectric 508 Superlattices. Phys. Rev. Lett. 58, 2486-2489. (doi:10.1103/PhysRevLett.58.2486)
- 509 37. González-Urbina L, Pérez-Moreno J, Clays K, Kolaric B. 2016 Phosphorescence
- 510 emission from BAlq by forced intersystem crossing in a colloidal photonic crystal, Mol. Phys.
- 511 114, 2248-2252. (doi:10.1080/00268976.2016.1194495)
- 38. González-Urbina L, Baert K, Kolaric B, Pérez-Moreno J, Clays K. 2012 Linear and
 Nonlinear Optical Properties of Colloidal Photonic Crystals. Chem. Rev. 112, 2268-2285.
 (doi:10.1021/cr200063f)
- 515 39. Kumazawa K, Tanaka S, Negita K, Tabata H. 1994 Fluorescence from Wing of *Morpho* 516 *sulkowskyi* Butterfly. Jpn. J. Appl. Phys. 33, 2119-2122. (doi:10.1143/JJAP.33.2119)
- 40. Lawrence C, Vukusic P, Sambles R. 2002 Grazing-incidence iridescence from a butterfly wing. Appl. Optics 41, 437-441. (doi:10.1364/AO.41.000437)
- 41. Vigneron JP, Kertész K, Vértesy Z, Rassart M, Lousse V, Bálint Zs, Biró LP. 2008
- 520 Correlated diffraction and fluorescence in the backscattering iridescence of the male butterfly
- 521 *Troides magellanus* (Papilionidae). Phys. Rev. E 78, 021903.
- 522 (doi:10.1103/PhysRevE.78.021903)

42. Van Hooijdonk E, Barthou C, Vigneron JP, Berthier S. 2011 Detailed experimental analysis of the structural fluorescence in the butterfly *Morpho sulkowskyi* (Nymphalidae). J. Nanophotonics 5, 053525. (doi:10.1117/1.3659147)

43. Van Hooijdonk E, Berthier S, Vigneron JP. 2012 Bio-inspired approach of the
fluorescence emission properties in the scarabaeid beetle *Hoplia coerulea* (Coleoptera):
Modeling by transfer-matrix optical simulations. J. Appl. Phys. 112, 114701.
(doi:10.1063/1.4768896)

44. Van Hooijdonk E, Vandenbem C, Berthier S, Vigneron JP. 2012 Bi-functional photonic
structure in the *Papilio nireus* (Papilionidae): modeling by scattering-matrix optical
simulations. Opt. Express 20, 22001-22011. (doi:10.1364/OE.20.022001)

45. Van Hooijdonk E, Barthou C, Vigneron JP, Berthier S. 2012 Angular dependence of structural fluorescent emission from the scales of the male butterfly *Troïdes magellanus* (Papilionidae). J. Opt. Soc. Am. B 29, 1104-1111. (doi:10.1364/JOSAB.29.001104)

46. Van Hooijdonk E, Berthier S, Vigneron JP. 2012 Contribution of both the upperside and
the underside of the wing on the iridescence in the male butterfly *Troïdes magellanus*(Papilionidae). J. Appl. Phys. 112, 074702. (doi:10.1063/1.4755796)

47. Van Hooijdonk E. 2012 Etude théorique et expérimentale de la fluorescence de

structures photoniques naturelles. Ph.D. thesis - Facultés Universitaires Notre-Dame de la
 Paix (FUNDP) and Université Pierre et Marie Curie (Paris VI), Namur & Paris.

48. Van Hooijdonk E, Barthou C, Vigneron JP, Berthier S. 2013 Yellow structurally modified fluorescence in the longhorn beetles *Celosterna pollinosa sulfurea* and *Phosphorus virescens* (Cerambycidae). J. Lumin. 136, 313-321. (doi:10.1016/j.jlumin.2012.12.022)

49. Mason CW. 1927 Structural Colors in Insects. II. J. Phys. Chem. 31, 321-354.
(doi:10.1021/j150273a001)

547 50. Vigneron JP, Colomer JF, Vigneron N, Lousse V. 2005 Natural layer-by-layer photonic 548 structure in the squamae of *Hoplia coerulea* (Coleoptera). Phys. Rev. E 72, 061904. 549 (doi:10.1103/PhysRevE.72.061904)

550 51. Deparis O, Mouchet SR, Dellieu L, Colomer JF, Sarrazin M. 2014 Nanostructured 551 surfaces: Bioinspiration for transparency, coloration and wettability. Mater. Today Proc. 1S, 552 122-129. (doi:10.1016/j.matpr.2014.09.008)

553 52. Vukusic P, Sambles JR, Lawrence CR, Wootton RJ. 1999 Quantified interference and

diffraction in single *Morpho* butterfly scales. Proc. R. Soc. Lond. B 266, 1403-1411.

555 (doi:10.1098/rspb.1999.0794)

556 53. Sollas IBJ. 1907 On the Identification of Chitin by Its Physical Constants. Proc. R. Soc. 557 Lond. B 79, 474-484. (doi:10.1098/rspb.1907.0042)

558 54. Berthier S, Charron E, Da Silva A. 2003 Determination of the cuticle index of the scales 559 of the iridescent butterfly *Morpho menelaus*. Opt. Commun. 228, 349-356. 560 (doi:10.1016/j.optcom.2003.10.032)

55. Leertouwer HL, Wilts BD, Stavenga DG. 2011 Refractive index and dispersion of butterfly chitin and bird keratin measured by polarizing interference microscopy. Opt. Express 19, 24061-24066. (doi:10.1364/OE.19.024061)

564 56. Yoshioka S, Kinoshita S. 2011 Direct determination of the refractive index of natural 565 multilayer systems. Phys. Rev. E 83, 051917. (doi: 10.1103/PhysRevE.83.051917)

566 57. Yeh P. 2005 Optical Waves in Layered Media. Wiley-Interscience, Hoboken.

567 58. Taflove A, Hagness SC. 2000 Computational Electrodynamics: The Finite-Difference 568 Time-Domain Method. Artech, Norwood.

569 59. Oskooi AF, Roundy D, Ibanescu M, Bermel P, Joannopoulos JD, Johnson SG. 2010

570 MEEP: A flexible free-software package for electromagnetic simulations by the FDTD

571 method. Comp. Phys. Commun. 181, 687-702. (doi:10.1016/j.cpc.2009.11.008)

- 572 60. Vandenbem C. 2006 Contribution à l'étude de la réflectance et du confinement des
 573 modes dans les systèmes optiques stratifiés. Ph.D. thesis Facultés Universitaires Notre574 Dame de la Paix (FUNDP), Namur.
- 575 61. Nikolaev IS, Vos WL, Koenderink AF. 2009 Accurate calculation of the local density of
- 576 optical states in inverse-opal photonic crystal. J. Opt. Soc. Am. B 26, 987-997.
- 577 (doi:10.1364/JOSAB.26.000987)
- 578 62. Deparis O, Vandenbern C, Rassart M, Welch VL, Vigneron JP. 2006 Color-selecting
- reflectors inspired from biological periodic multilayer structures. Opt. Express 14, 3547-3555.
 (doi:10.1364/OE.14.003547)
- 581 63. Barnes WL. 1998 Fluorescence near interfaces: the role of photonic mode density. J.
- 582 Modern Opt. 45, 661-699. (doi:10.1080/09500349808230614)
- 583 64. Kolaric B, Desprez S, Brau F, Damman P. 2012 Design of curved photonic crystal using
- swelling induced instabilities. J. Mater. Chem. 22, 16205-16208. (doi:10.1039/C2JM32997B)
 585

Submitted to Proceedings of the Royal Society B: For Review Only

586	
587	Figure 1 The male H. coerulea beetle displays a vivid violet-blue iridescent colour (a) due to a porous multilayer structure
588	located in the scales covering its elytra and thorax. The photonic structure is a periodic stack of thin pure cuticle layers and
589	mixed air-cuticle porous layers (b,c). In the structural model used for simulations, the layers comprising a mixture of air (in the
590	pores) and cuticle material (associated with the rods) were approximated by homogeneous layers with an effective refractive
591	index (RI) (d).

- 592 **Figure 2** Colour and fluorescence changes of the scales of the male *H. coerulea* beetle induced by contact with water.
- 593 Illuminated by visible white light (at normal incidence and detection), the beetle scales appear violet-blue in the dry state (a) and
- 594 green in the wet state (b). The reflectance peak wavelength shifts from 458 nm to 525 nm when the elytron is in contact with
- 595 water (c). Under UV light (with a 45° incidence and detection angle), the scales produce a turquoise coloured emission in the
- 596 dry state (d) and a dark blue colour in the wet state (e). Where scales overlap, the fluorescence intensity is higher. This effect is
- 597 due to the transparency of the scales at the emitted wavelengths. Although there is almost no water-induced change in the
- 598 excitation spectrum peak wavelength and its associated FWHM, the emission spectrum peak wavelength shifts from 463 nm to
- 599 446 nm when the elytron is in contact with water. The associated FWHM reduces from 121 nm to 105 nm (f).

600	Figure 3 Reflectance spectra calculated for the modelled photonic structure of a male H. coerulea beetle (Figure 1c) using
601	unpolarised light at normal incidence. The reflectance peak centre wavelength shifts from 461 nm to 501 nm when the structure
602	changes from dry (blue curves) to wet (green curve) state and the reflectance peak intensity decrease (a). The related photonic
603	band structure and density of optical states (DOS) are also modified accordingly (b and c). The reflectance peak widths and the
604	PhBG widths decrease in the wet state.

- Figure 4 When the pores of the modelled photonic structure are filled with water, the peak emission wavelength shifts from 480 nm to 428 nm (a) and from 429 nm to 416 nm (b) in the cases of both investigated models. a) In the first model, emitting planar sources are assumed to be located in the different pure cuticle layers of the photonic structure. The presented spectra result from the averages over 12 simulated spectra that individually correspond to fluorophores located in each of the 12 pure cuticle layers. b) In the second model, the fluorophores are assumed to be 180 point sources distributed across the photonic structure.
- 611

612

		Dry state		Wet state	
	Emission wavelength (nm)	Decay time τ (ns)	Standard error (SE) (ns)	Decay time τ (ns)	Standard error (SE) (ns)
	466	3.9	0.55	1.4	0.04
		0.79	0.02		
	546	1.9	0.05	1.4	0.04
613	Table 1 Decay times a	nd the related standard er	rors of the fluorophores en	nbedded in <i>H. coerulea</i> be	etle's scales in both dry
614	and wet states inside (4	166 nm) and outside (546	nm) the PhBG of the struct	ture. The incident light form	ned a 45° angle with the

615 direction normal to the sample surface, with a wavelength equal to 376 nm. The emitted light was detected at a 45° angle on the

616

other side of the normal direction.



The male *H. coerulea* beetle displays a vivid violet-blue iridescent colour (a) due to a porous multilayer structure located in the scales covering its elytra and thorax. The photonic structure is a periodic stack of thin pure cuticle layers and mixed air-cuticle porous layers (b,c). In the structural model used for simulations, the layers comprising a mixture of air (in the pores) and cuticle material (associated with the rods) were approximated by homogeneous layers with an effective refractive index (RI) (d). Figure 1

527x417mm (120 x 120 DPI)



Colour and fluorescence changes of the scales of the male *H. coerulea* beetle induced by contact with water. Illuminated by visible white light (at normal incidence and detection), the beetle scales appear violet-blue in the dry state (a) and green in the wet state (b). The reflectance peak wavelength shifts from 458 nm to 525 nm when the elytron is in contact with water (c). Under UV light (with a 45° incidence and detection angle), the scales produce a turquoise coloured emission in the dry state (d) and a dark blue colour in the wet state (e). Where scales overlap, the fluorescence intensity is higher. This effect is due to the transparency of the

scales at the emitted wavelengths. Although there is almost no water-induced change in the excitation spectrum peak wavelength and its associated FWHM, the emission spectrum peak wavelength shifts from 463 nm to 446 nm when the elytron is in contact with water. The associated FWHM reduces from 121 nm to

105 nm (f). Figure 2 401x222mm (120 x 120 DPI)



Reflectance spectra calculated for the modelled photonic structure of a male *H. coerulea* beetle (Figure 1c) using unpolarised light at normal incidence. The reflectance peak centre wavelength shifts from 461 nm to 501 nm when the structure changes from dry (blue curves) to wet (green curve) state and the reflectance peak intensity decrease (a). The related photonic band structure and density of optical states (DOS) are also modified accordingly (b and c). The reflectance peak widths and the PhBG widths decrease in the wet state. Figure 3

194x237mm (120 x 120 DPI)



When the pores of the modelled photonic structure are filled with water, the peak emission wavelength shifts from 480 nm to 428 nm (a) and from 429 nm to 416 nm (b) in the cases of both investigated models. a) In the first model, emitting planar sources are assumed to be located in the different pure cuticle layers of the photonic structure. The presented spectra result from the averages over 12 simulated spectra that individually correspond to fluorophores located in each of the 12 pure cuticle layers. b) In the second model, the fluorophores are assumed to be 180 point sources distributed across the photonic structure. Figure 4

375x179mm (120 x 120 DPI)